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# Saliva Parameters and Erosive Wear in Adolescents

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## Key Words

Demineralization · Saliva · Tooth wear

## Abstract

The aim of this study was to investigate the relationship between several parameters of saliva and erosive wear in adolescents. (Un-)stimulated saliva was collected from 88 adolescents with erosion and 49 controls (age  $16 \pm 1$  years). Flow rate, pH and buffer capacity were determined immediately. Total protein content, carbonic anhydrase VI, amylase, albumin, calcium, phosphate, urea, sodium, chloride and potassium were measured at a later time. Unstimulated flow rate was found to be significantly lower in subjects with erosive wear ( $p = 0.016$ ). The chloride concentration in unstimulated saliva was found to be significantly higher in the erosion group ( $p = 0.019$ ).

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Erosive tooth wear is the accelerated loss of dental hard tissue through the combined effect of erosion and mechanical wear. Erosion is defined as partial demineralization of enamel or dentine by intrinsic or extrinsic acids. The reported prevalence of erosive wear in adolescents

ranges between 11 and 100% [Jaeggi and Lussi, 2006]. In a population in Oss, The Netherlands, 30% of the 11-year-old children showed signs of erosive wear. At age 15 this prevalence increased to 44% [El Aidi et al., 2010]. Erosive wear starts at a young age and, if unchecked, may compromise the long-term prognosis of the dentition; therefore early diagnosis and prevention of erosive tooth wear is necessary.

The best way to limit dental erosion is to prevent contact between acids and tooth tissue. The role of saliva in this process is still not fully clear. Saliva can protect tooth tissue in several ways. Factors like salivary flow, pellicle and buffer capacity contribute to this protective role of saliva. After intake of acidic food or drinks saliva acts as a diluting agent, clearing the remnants of acids in the mouth. In earlier research it was found that a high salivary flow rate results in a better clearance of acids in the mouth [Järvinen et al., 1991].

The pellicle is a layer of proteins, which is derived from saliva and can form a diffusion barrier. The pellicle is believed to protect the enamel and dentine against erosion [Wetton et al., 2007; Jager et al., 2011; White et al., 2012]. The formation of pellicle is influenced by several factors: the protein content in the organic matrix of enamel itself, and therefore its electronic charge [Lubarsky et al., 2012]

and the availability of proteins in saliva [Nieuw Amerongen, 2004]. This pellicle of adsorbed salivary proteins might act as a diffusion barrier or a selective permeable membrane reducing direct contact between acids and tooth surface and thus reducing demineralization of the surface. The proteins that form the pellicle affect its functions such as ion transport potential, regulation of calcium phosphate crystallization and bacterial adherence [Hannig and Joiner, 2006]. One protein of interest is albumin. It has been stated that in the presence of albumin, calcium and phosphate dissolve faster in an aqueous environment [Kandori et al., 2005]. Others found no significant relationship between the presence of albumin and demineralization of enamel [Kielbassa et al., 2005].

Saliva also acts as a buffering agent. Concentrations of amylase, carbonic anhydrase VI (CA-6), bicarbonate, phosphate and urea can influence the buffering capacity [Humphrey and Williamson, 2001; Nieuw Amerongen, 2004]. For instance, CA-6 collaborates with the bicarbonate buffer system. CA-6 is believed to be present in enamel pellicle [Leinonen et al., 1999]. An *in situ* study showed a significant negative correlation between the concentration of CA-6 and the loss of calcium from hydroxyapatite discs [unpubl. data], showing that CA-6 may play a role in the prevention of erosive wear.

Several components of saliva have been discussed in relation to the salivary pellicle and buffer capacity, such as minerals like calcium, potassium, sodium and chloride. Calcium, like phosphate, is important in the equilibrium between enamel mineral and the surrounding oral fluids. Potassium has been shown, as K-citrate in a dentifrice, to be effective against erosive wear *in vitro* [Kato et al., 2010]. Concentrations of sodium and chloride in saliva both have been found to be negatively related to loss of calcium from hydroxyapatite due to an erosive challenge [Jager et al., 2011].

Available information about the association between quantitative and qualitative aspects of saliva and erosive wear is very limited. It was the aim of this study to investigate this association in adolescents with early erosive wear.

## Subjects and Methods

The subjects in this study were regular attendants of the paediatric dental practice Jeugd tandverzorging in Oss, The Netherlands. A longitudinal study was performed between 2005 and 2008 among 10- to 12-year-old children. In this study the prevalence and incidence of erosive tooth wear were determined among 572 children [El Aidi et al., 2010]. The erosion index of Lussi [1996],

modified by van Rijkom [2002], was used. This index scores the amount of visible erosion between 0 and 3, score 0 representing no visible erosion; score 1 representing slight erosion of enamel, silky-melted appearance surface; score 2 representing severe erosion of enamel, light-yellow surface, and score 3 representing dentine erosion, dark-yellow surface.

A subsample of this population was selected, based on a feasibility criterion: the children still needed to be enrolled as patients at Jeugd tandverzorging. METC consent was obtained (CEOM No. 2003-207). The selected sample consisted of 200 subjects. The erosion scores at the end of the longitudinal study ( $\pm 12$  months previously) were distributed as follows: score 0 ( $n = 68$ ), score 1 ( $n = 57$ ), score 2 ( $n = 37$ ), score 3 ( $n = 38$ ). The subsample for controls (score 0) was randomly selected, the subjects with erosion were selected based on the severity score as estimated in the previous study [El Aidi et al., 2010]. The age of the children was  $16 \pm 1$  years. Informed consent of the parents and the children was obtained.

### Collection and Analysis of Saliva

The children were asked not to eat or drink 1 h before their appointment (always between 1 and 5 p.m.). This was checked by asking the subjects before saliva collection. From subjects eating or drinking 1 h before their appointment only stimulated saliva was collected. First, unstimulated whole saliva was collected. Children were asked to spit in a cup until approximately 5 ml was obtained. The subjects were instructed not to 'actively obtain' saliva and not to speak during collection of unstimulated saliva. Subsequently, children chewed on a piece of Parafilm (Parafilm M, Pechiney Plastic Packaging Co., Chicago, Ill., USA) to obtain stimulated whole saliva. Time to collect approximately 5 ml saliva was noted. Before and after collection the cups were weighed. Flow rate, pH and buffer capacity were immediately determined. The pH was estimated with a calibrated pH electrode (pH electrode WTW Sentix 61, Weilheim, Germany). Buffer capacity was measured as the drop in pH when adding 0.75 ml 0.5 mM HCl solution to the same amount of saliva. The remaining saliva was centrifuged for 5 min at 10,000 *g* to remove debris. The centrifuged samples were stored on ice (maximum 7 h) until late afternoon when they were frozen in liquid nitrogen at  $-80^{\circ}\text{C}$ . Analysis of saliva was conducted at a later moment. Calcium, phosphate, sodium, chloride, urea, albumin, amylase and total protein concentration were analysed using similar methods as in a previous publication [Jager et al., 2011]. The concentration of CA-6 in unstimulated and stimulated whole saliva was determined using an enzyme-linked immunosorbent assay (ELISA). Sheep anti-rabbit CA-6 antibody, diluted 1:10,000, was coated overnight on 96-well ELISA plates in pH 9.6 carbonate buffer at  $4^{\circ}\text{C}$ . After this incubation period the plate was washed 3 times with phosphate-buffered saline (PBS)-Tween 20 buffer solution (0.1 M PBS, 0.5% Tween 20, pH 7.2 diluted 1:10). To obtain human CA-6 standards, CA-6 was purified from the parotid saliva of 4 donors by inhibitor affinity chromatography [Murakami and Sly, 1987]. The stimulated parotid saliva was collected with Lashley cups while the donors sucked on sugar-free candy.

The saliva samples and the purified CA-6 standards (both diluted 1:50 in PBS-Tween 20) were added to the wells (100  $\mu\text{l}$  of diluted standard or sample) and diluted 4 times down the plate. The mixture was incubated for 2 h at  $37^{\circ}\text{C}$  and after incubation the plate was washed again 3 times and a second antibody (100  $\mu\text{l}$  of 1:1,000 goat anti-human CA-6 in PBS-Tween 20) was added to each well. After another 2-hour incubation at  $37^{\circ}\text{C}$  the plates were

**Table 1.** Unstimulated saliva: erosion versus controls

	Erosion			Controls			p value
	n	median	interquartile range	n	median	interquartile range	
pH	82	6.91	6.68–7.05	46	6.93	6.78–7.14	0.173
Buffer capacity	82	6.10	5.70–6.38	46	6.10	5.75–6.41	0.825
Flow, ml/min	82	0.50	0.31–0.71	46	0.68	0.48–0.86	0.016*
Albumin, mg/l	81	92	56–157	44	70	46–123	0.411
Amylase, units/l	81	132,271	86,289–183,480	44	138,952	94,472–201,695	0.554
CA-6	81	29.3	14.2–44.8	44	29.9	14.4–59.25	0.640
Total protein, g/l	81	0.4	0.3–0.6	44	0.4	0.3–0.6	0.579
Calcium, mmol/l	81	1.1	0.9–1.2	44	1.05	0.9–1.2	0.593
Phosphate, mmol/l	81	5.8	4.9–7.1	44	5.6	4.4–6.8	0.242
Urea, mmol/l	81	5	4–6	44	5	4–6	0.536
Sodium, mmol/l	81	6	4–9	45	6	3–7	0.219
Chloride, mmol/l	81	21	18–24	44	19.5	17–21.25	0.019*
Potassium, mmol/l	81	21.5	18.2–24.2	45	20.8	18.2–22.8	0.263

\* Significant.

washed 3 times with PBS-Tween and then incubated with horse-radish peroxidase-labelled anti-goat (100 µl) diluted 1:2,000 in PBS-Tween 20, for a further hour at 37°C. After three more washes with PBS-Tween the substrate was added. This consisted of 0.5 ml of tetramethylbenzidine stock solution (3 mg/ml in DMSO) and 5 µl of 3% hydrogen peroxide in 20 ml of sodium acetate buffer (100 mM, pH 5.5). The reaction was stopped after 3 min by the addition of 50 µl of 2 M sulphuric acid and the absorbance was read at 450 nm in a microplate absorbance reader (model 168-1130XTU, Bio-Rad Laboratories, Hemel Hempstead, UK). The concentration of CA-6 was expressed in micrograms per millilitre.

#### Statistical Analysis

The data were analysed with SPSS (PSAW Statistics release 18.0.2, SPSS Inc., Chicago, Ill., USA). Children with scores of 1, 2 or 3 were merged into one group, the erosion group. The control group consisted of children with score 0, no visible erosion. Because of the skewed distribution of the data, differences between the groups were tested with a Mann-Whitney U test. Significant diet factors related to dental erosion were evaluated in a previous study [El Aidi et al., 2011]. To test for confounding, these factors were added to a multivariate logistic regression model. A p value less than 0.05 was considered statistically significant.

## Results

Of the 200 invited children, 63 did not participate in this study. Some appeared to have moved to another city and others did not show up at their appointment (even after a second appointment). Of these children, 44 (33%) were from the erosion group and 19 (28%) were controls. During data collection, some children reported having

had lunch shortly before their appointment; others were chewing gum at arrival. Therefore it was not possible to collect unstimulated saliva from these subjects. One subject suffered from hyposalivation, another saliva sample was contaminated with blood. Occasionally, samples were labelled accidentally as stimulated or unstimulated saliva, or there was not enough saliva available for analysis. This explains the slight differences in the number of subjects in tables 1 and 2. In table 1 the median and interquartile range of the different variables were listed for unstimulated saliva, in table 2 for stimulated saliva. The results of the Mann-Whitney U test are also presented in both tables.

Only two significant differences between the erosion and the control group were observed, both in unstimulated saliva. The flow rate of unstimulated saliva was significantly lower ( $p = 0.016$ ) in subjects with erosion: 0.50 ml/min in the erosion group versus 0.68 ml/min in the control group. The concentration of chloride in unstimulated saliva was significantly higher in subjects with erosion ( $p = 0.019$ ): 21 mmol/ml in the erosion group versus 19.5 mmol/ml in the control group. There was a non-significant ( $p = 0.072$ ) trend for amount of total protein in stimulated saliva. In subjects with erosion the amount of protein (0.3 g/l) was lower than in the control group (0.4 g/l).

**Table 2.** Stimulated saliva: erosion versus controls

	Erosion			Controls			p value
	n	median	interquartile range	n	median	interquartile range	
pH	86	7.18	7.01–7.32	49	7.25	7.09–7.37	0.150
Buffer capacity	86	6.54	6.25–6.74	49	6.54	6.39–6.73	0.771
Flow, ml/min	86	1.18	0.96–1.73	49	1.30	0.99–1.79	0.313
Albumin, mg/l	85	53	35–75	49	52	36–84	0.419
Amylase, units/l	85	138,951	103,850–182,340	47	147,109	114,023–225,450	0.137
CA-6	84	27.2	14.9–44.2	47	34.11	19.9–58.1	0.175
Total protein, g/l	85	0.3	0.2–0.4	48	0.4	0.3–0.5	0.072
Calcium, mmol/l	85	0.9	0.8–1.1	49	0.9	0.8–1.1	0.754
Phosphate, mmol/l	85	4.6	3.8–5.6	48	4.5	4.1–5.2	0.901
Urea, mmol/l	85	4	3–4	48	4	3–5	0.798
Sodium, mmol/l	85	6	4–9	49	6	4–9	0.587
Chloride, mmol/l	85	19	18–22	49	20	18–22	0.691
Potassium, mmol/l	85	19.8	17.3–22.5	49	20.6	18.8–22.1	0.209

## Discussion

A lower unstimulated salivary flow rate was found in subjects with erosive tooth wear. Earlier studies showed varying results concerning the unstimulated flow rate. Järvinen et al. [1991] reported a 5 times higher chance of developing dental erosion when the flow rate of unstimulated saliva was less than 0.1 ml/min. Other studies were not able to find an effect of unstimulated flow rate [O'Sullivan and Curzon, 2000; Johansson et al., 2002; El Aidi et al., 2011]. However, the circumstances in the above-mentioned studies were quite variable. For instance, using different diagnostic methods more or less specific for erosive wear may have influenced the outcomes of these studies.

A higher concentration of chloride in unstimulated saliva in subjects with erosion has not been reported yet. On the contrary, a few reports point in the opposite direction. An in vitro/in situ study reported reduction of erosion when the concentration of chloride in stimulated saliva increased [Jager et al., 2011]. Another in vitro study showed that, also in the presence of NaCl solution, less erosion of hydroxyapatite takes place. The authors attribute this protective effect to sodium [Kwon et al., 2009]. It is known that the concentration of chloride in saliva is related to the concentration of sodium [Ferguson et al., 1973]. Both concentrations are also related to salivary flow rate. If the flow rate is higher, less time for re-sorption of minerals is available, so saliva will contain more chloride and sodium. This contrast and the fact, explained later, that the chloride effect disappears when a

subgroup is analysed in our study lead us to suspect that the result is a chance finding.

Diet is an important aetiological factor in the development of dental erosion. The subjects in this study had all participated in a longitudinal study in which many factors were evaluated and analysed [El Aidi et al., 2011]. To test for confounding in our study, food products with a significant relation to the incidence of erosion were tested for significant correlation with respect to unstimulated flow rate and chloride concentration. The unstimulated chloride concentration was significantly correlated to the use of alcoholic mixed drinks and fruit drinks. When these factors were put in a multivariate model the unstimulated chloride concentration was significantly related to having dental erosion. Therefore one could conclude that the unstimulated flow rate and the chloride concentration are independent factors contributing to dental erosion.

The subjects had to spit in a cup until 5 ml of saliva was collected. The advantage of this method is that there is always enough saliva for analysis. On the other hand, due to vaporization of CO<sub>2</sub>, the pH of saliva may rise [Bardow et al., 2000; Schipper et al., 2007]. This rise in pH is caused by the presence of the carbonic acid equilibrium in saliva ( $\text{HCO}_3^- + \text{H}^+ \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{H}_2\text{O} + \text{CO}_2$ ). When CO<sub>2</sub> dissolves, the equilibrium will move to the right side, and therefore the amount of H<sup>+</sup> will decrease, causing an increase in salivary pH. Not all the subjects collected 5 ml in the same time. The pH and buffer capacity of the saliva samples may have been influenced by this effect, and those results need to be viewed with some care.



Of the 137 participating subjects, 36 (27% erosion group, 24% control group) brushed their teeth less than 1 h before saliva was collected, because of the appointment that would follow for their biannual check-up. When we analysed the data without these subjects, the unstimulated flow rate remained significantly different. The significant association with chloride disappeared but an association with calcium concentration in stimulated saliva appeared. Because of the high number of parameters tested in this study relative to the study population, chance effects are more likely to occur.

In the study we found only very limited differences between saliva of children with and without erosive tooth wear. Only for the flow rate of unstimulated saliva a ro-

bust association could be found. The aetiology of erosive tooth wear is multifactorial. Saliva, pellicle, diet and drinking habits and mechanical wear are all factors influencing the clinical presentation of dental erosion. We suggest that a larger sample size and perhaps more advanced stages of erosive wear will be required to establish more precisely the role of saliva in the process of erosive tooth wear in adolescents.

## Disclosure Statement

There exist no conflicts of interest.

## References

- Bardow A, Moe D, Nyvad B, Nauntofte B: The buffer capacity and buffer systems of human whole saliva measured without loss of CO<sub>2</sub>. *Arch Oral Biol* 2000;45:1–12.
- El Aidi H, Bronkhorst EM, Huysmans MC, Truin GJ: Multifactorial analysis of factors associated with the incidence and progression of erosive tooth wear. *Caries Res* 2011;45:303–312.
- El Aidi H, Bronkhorst EM, Huysmans MCDNJM, Truin GJ: Dynamics of tooth erosion in adolescents: a 3-year longitudinal study. *J Dent* 2010;38:131–137.
- Ferguson DB, Fort A, Elliot AL, Potts AJ: Circadian rhythms in human parotid saliva flow rate and composition. *Arch Oral Biol* 1973;18:1155–1173.
- Hannig M, Joiner A: The structure, function and properties of the acquired pellicle. *Monogr Oral Sci* 2006;19:29–64.
- Humphrey SP, Williamson RT: A review of saliva: normal composition, flow and function. *J Prosthet Dent* 2001;85:162–169.
- Jaeggi T, Lussi A: Prevalence, incidence and distribution of erosion. *Monogr Oral Sci* 2006;20:44–65.
- Jager DHJ, Vieira AM, Ligtenberg AJM, Bronkhorst EM, Huysmans MCDNJM, Vissink A: The effect of saliva on the susceptibility of hydroxyapatite to erosion. *Caries Res* 2011;45:532–537.
- Järvinen VK, Rytömaa II, Heinonen OP: Risk factors in dental erosion in children. *J Dent Res* 1991;70:942–947.
- Johansson AK, Lingström P, Birkhed D: Comparison of factors potentially related to the occurrence of dental erosion in high- and low-erosion groups. *Eur J Oral Sci* 2002;110:204–211.
- Kandori K, Masunari A, Ishikawa T: Study of adsorption mechanism of proteins onto synthetic calcium hydroxyapatites through ionic concentration measurements. *Calcif Tissue Int* 2005;76:194–206.
- Kato MT, Lancia M, Sales-Peres SH, Buzalaf MA: Preventive effect of commercial desensitizing toothpastes on bovine enamel erosion in vitro. *Caries Res* 2010;44:85–89.
- Kielbassa AM, Oeschger U, Schulte-Monting J, Meyer-Lueckel H: Microradiographic study on the effects of salivary proteins on in vitro demineralization of bovine enamel. *J Oral Rehabil* 2005;32:90–96.
- Kwon KY, Wang E, Chung A, Chang N, Lee SW: Effect of salinity on hydroxyapatite dissolution studied by atomic force microscopy. *J Phys Chem C* 2009;113:3369–3372.
- Leinonen J, Kivelä J, Parkkila S, Parkkila AK, Rajaniemi H: Salivary carbonic anhydrase isoenzyme VI is located in the human enamel pellicle. *Caries Res* 1999;33:185–190.
- Lubarsky GV, D'Sa RA, Deb S, Meenan BJ, Lemoine P: The role of enamel proteins in protecting mature human enamel against acidic environments: a double layer force spectroscopy study. *Biointerphases* 2012;7:14.
- Lussi A: Dental erosion clinical diagnosis and case history taking. *Eur J Oral Sci* 1996;104:191–198.
- Murakami H, Sly WS: Purification and characterization of human salivary carbonic anhydrase. *J Biol Chem* 1987;262:1382–1388.
- Nieuw Amerongen A: *Speekel, speekselklieren en mondgezondheid*. Houten, Bohn Stafleu van Loghum, 2004.
- O'Sullivan EA, Curzon MEJ: Salivary factors affecting dental erosion in children. *Caries Res* 2000;34:82–87.
- Schipper RG, Silletti E, Vingerhoeds MH: Saliva as research material: biochemical, physiochemical and practical aspects. *Arch Oral Biol* 2007;52:1114–1135.
- van Rijkom HM, Truin GJ, Frencken JEFM, König KG, van 't Hof MA, Bronkhorst EM, Roeters FJM: Prevalence, distribution and background variables of smooth-bordered tooth wear in teenagers in The Hague, The Netherlands. *Caries Res* 2002;36:147–154.
- Wetton S, Hughes J, Newcombe RG, Addy M: The effect of saliva derived from different individuals on the erosion of enamel and dentine: a study in vitro. *Caries Res* 2007;41:323–327.
- White AJ, Jones SB, Barbour ME, Churchley DR, Gracia LH, Rees GD: Inhibition of erosive dissolution by sodium fluoride: evidence for a dose-response. *J Dent* 2012;40:654–660.